

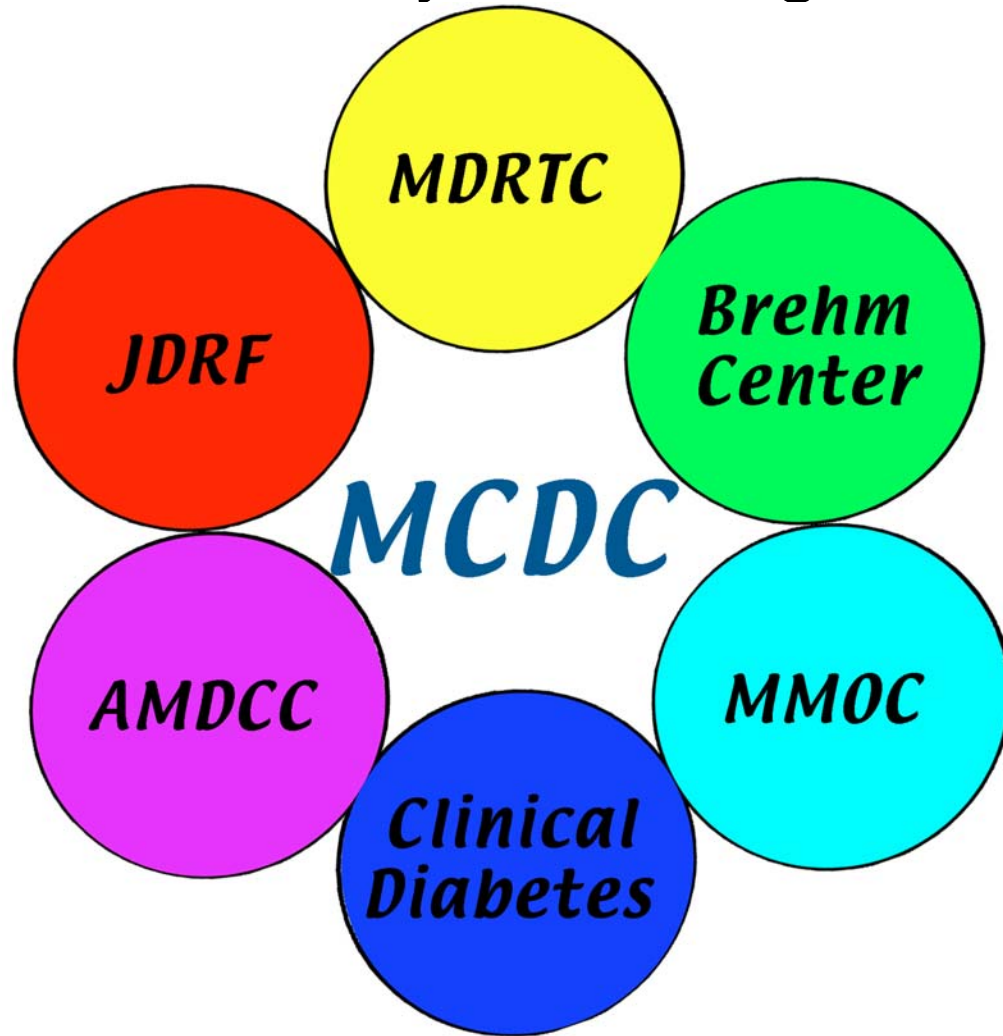
# Transcriptional regulatory networks in diabetic complications

Eva Feldman, Sub Pennathur and  
Matthias Kretzler

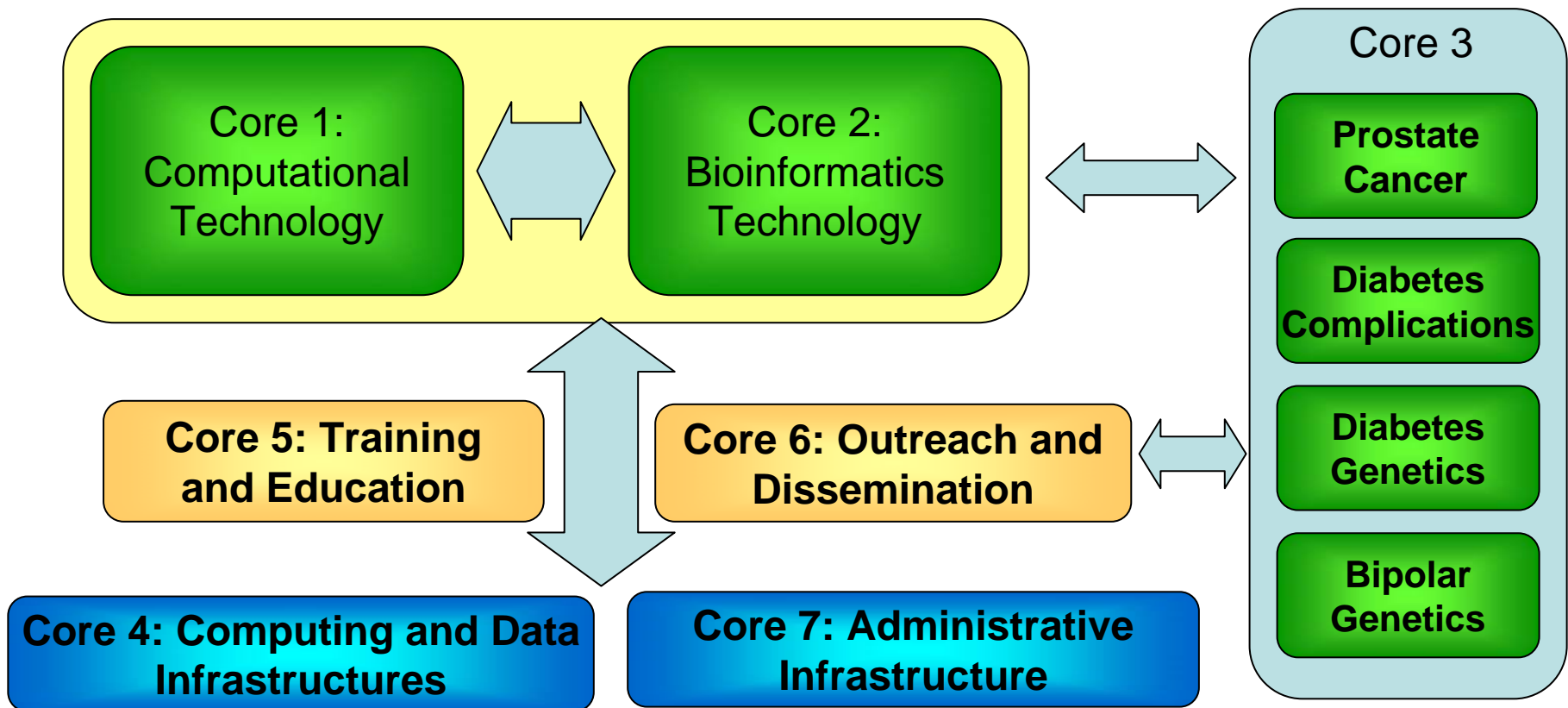
Departments of Neurology and Internal Medicine  
University of Michigan Medical Center



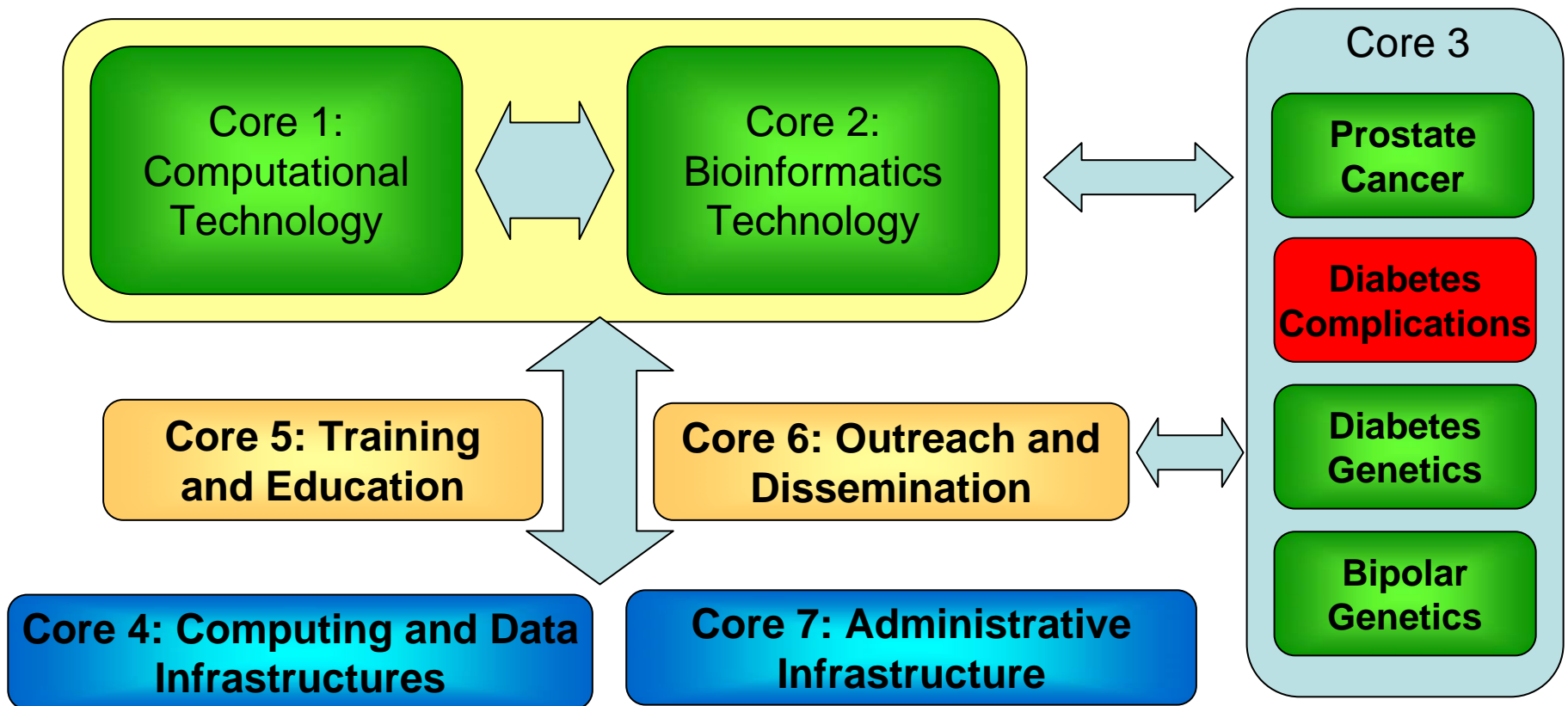
# Diabetes research environment at the University of Michigan



# NCIBI Structure



# NCIBI Structure



# Diabetic complications:

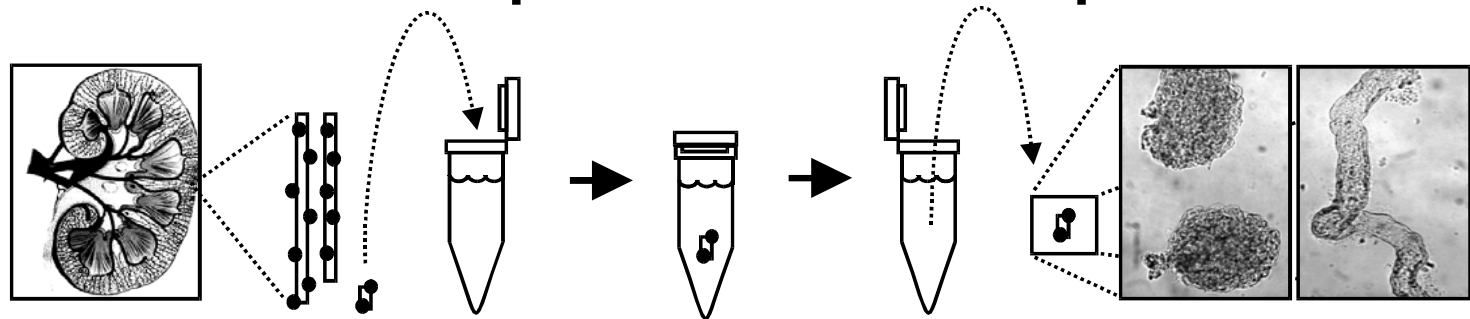
Tissue based diagnosis in nephrology  
enables molecular analysis of human disease

Harness genome wide profiling strategies for  
disease management of diabetic nephropathy

1. Develop and implement a tissue procurement protocol
2. Generate an expression map of renal disease
3. Extract relevant disease markers and pathways
4. Develop strategies for optimal data exploitation and clinical implementation



# 1. Tissue procurement protocol



Biopsy

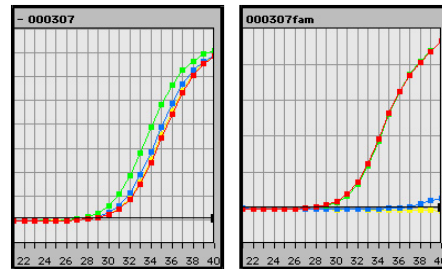
Storage

Dissection

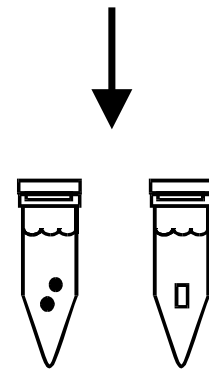


Clinical

Parameter



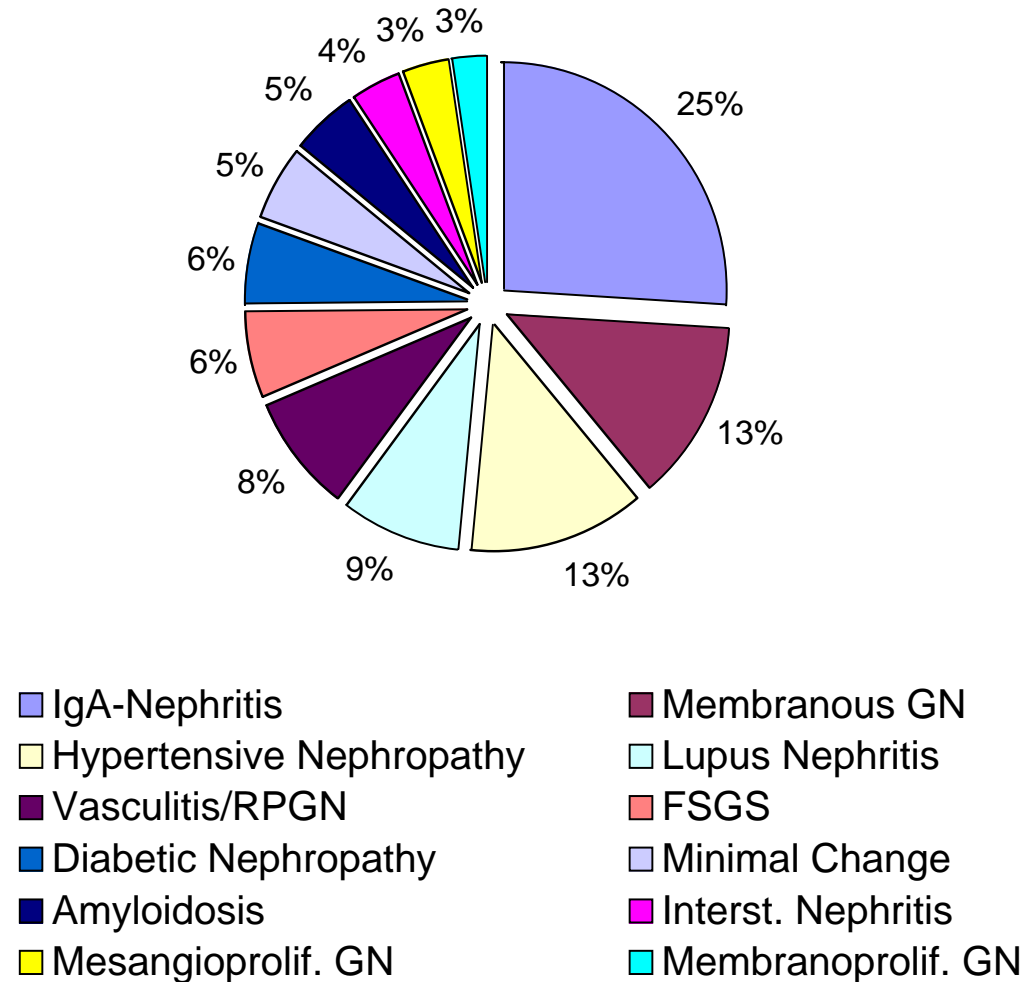
Expression-Data



RNA,  
cDNA

# Kidney biopsy bank:

2000 samples processed, QC passed, available for molecular analysis



## 2. Gene-expression map of human renal disease

- Human renal biopsies
  - Micro-dissected glomerular and tubulo-interstitial compartments
  - Linear amplification
  - Hybridization of RNA on human Affymetrix chips HG\_U133 A+B
    - >300 samples hybridized
- Disease categories
  - Living related donor pretransplant-biopsies (LD, n=13)
  - Cadaver donor pretransplant-biopsies (CD, n=4)
  - Tumor nephrectomies (TN, n=5)
  - Diabetic Nephropathy (DN, n=41)
  - Thin membrane disease (TMD, n=5)
  - Minimal change disease (MCD, n=12)
  - Hypertensive Nephropathy (HN, n=20)
  - IgA-Nephropathy (IgA, n=27)
  - Lupus-Nephritis (SLE, n=37)
  - Membranous Nephropathy (MGN, n=29)
  - FSGS (FSGS, n=14)



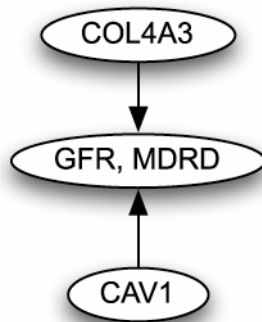


# 3. Extraction of diabetic disease markers and pathways

- Molecular diagnostic markers
- Transcriptional control of diabetic nephropathy
  - Transcriptional control mechanism of cell type specific gene expression
  - Identification of disease specific transcriptional control

# Molecular marker of diabetic end-organ damage

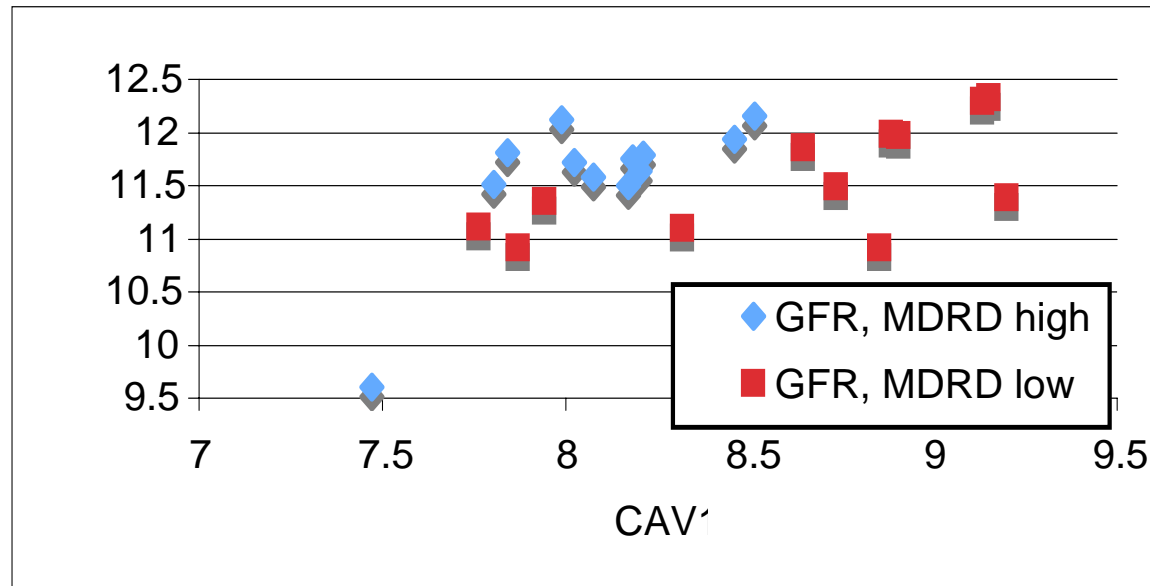
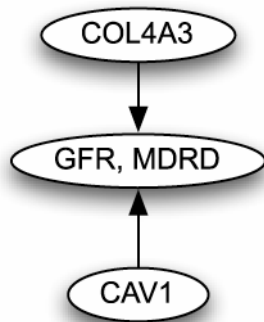
- Predicting clinical outcome in diabetic nephropathy: Bayesian network (P.Woolf):
  - Input (Parent)
    - RNA fingerprints in early human diabetic glomerulopathy
  - Output (Child)
    - Impairment of renal function
      - (Glomerular filtration rate, (GFR), MDRD formula)



# Molecular marker of diabetic end-organ damage

- Predicting clinical outcome in diabetic nephropathy: Bayesian network (P.Woolf):

- Input (Parent)
  - RNA fingerprints in early human diabetic glomerulopathy
- Output (Child)
  - Impairment of renal function
    - (Glomerular filtration rate, (GFR), MDRD formula)



- Collagen IV alpha 3 and Caveolin-1 relationship to GFR:
  - Key molecules for intact filter in animal models
  - Candidate predictors for non-invasive screening

# 3. Extraction of diabetic disease markers and pathways

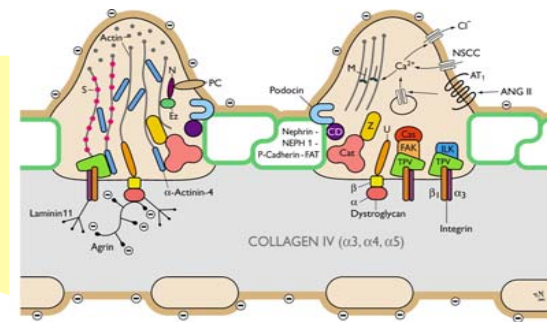
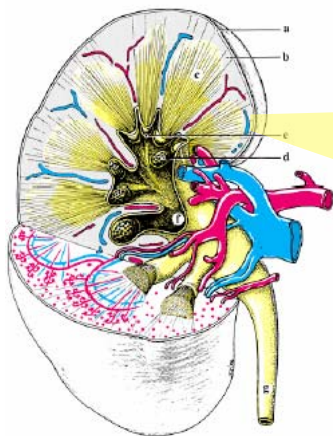
- Molecular diagnostic markers
- Transcriptional control of diabetic nephropathy
  - Transcriptional control mechanism of cell type specific gene expression
  - Identification of disease specific transcriptional control

# Filtration barrier failure in diabetic nephropathy: What defines who is expressed where?



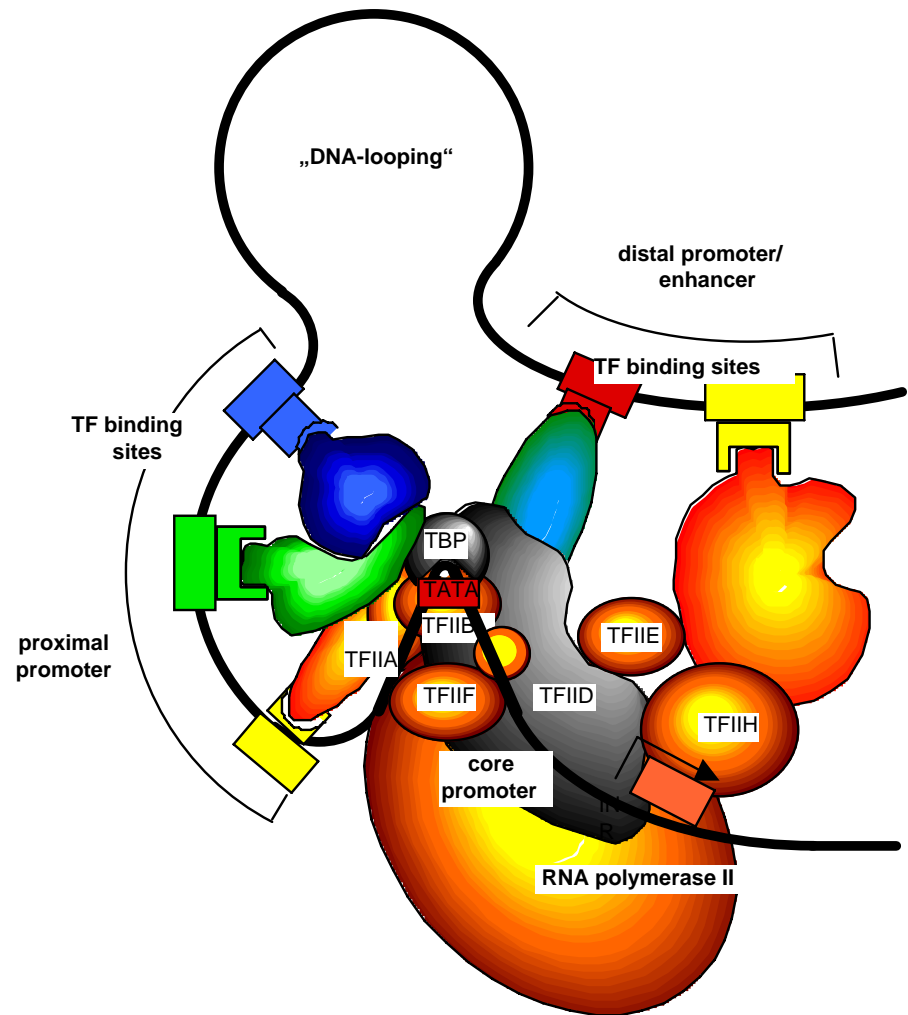
# Hypothesis: Promoter Co-regulation

- Gene function is not entirely a consequence of the amino acid sequence encoded by the gene
- Functional context of expression is an important component of "gene function"
- Promoter sequence determines gene regulation and contains information on this "context" of expression
- It may be possible to use features of promoters to elucidate information about the functional context of genes in silico

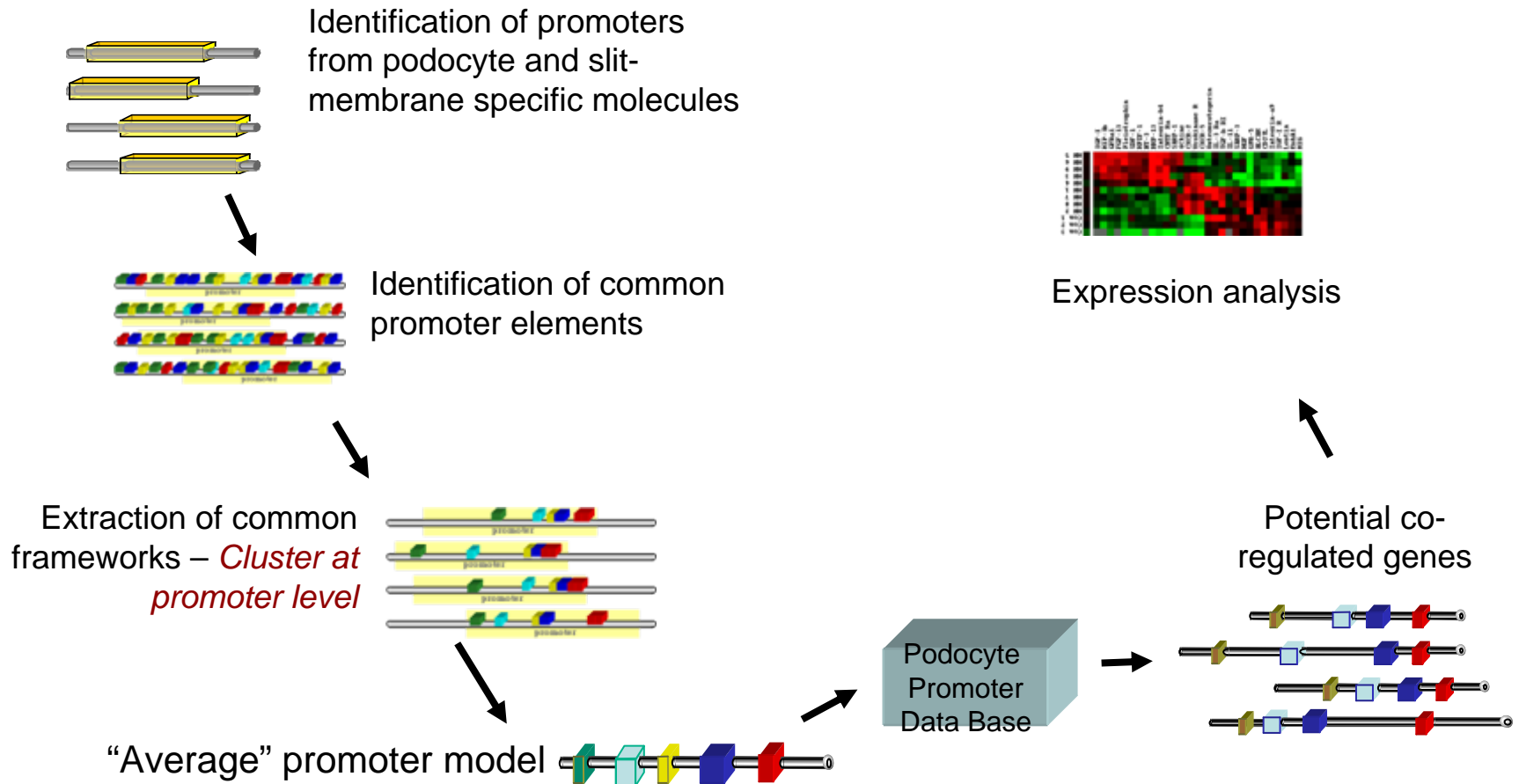


# Promoters show a unique three dimensional structure

- The order, spacing and orientation of TF sites are crucial for the structure of the active promoter
- Co-regulated promoters show similar TF site (module) organization



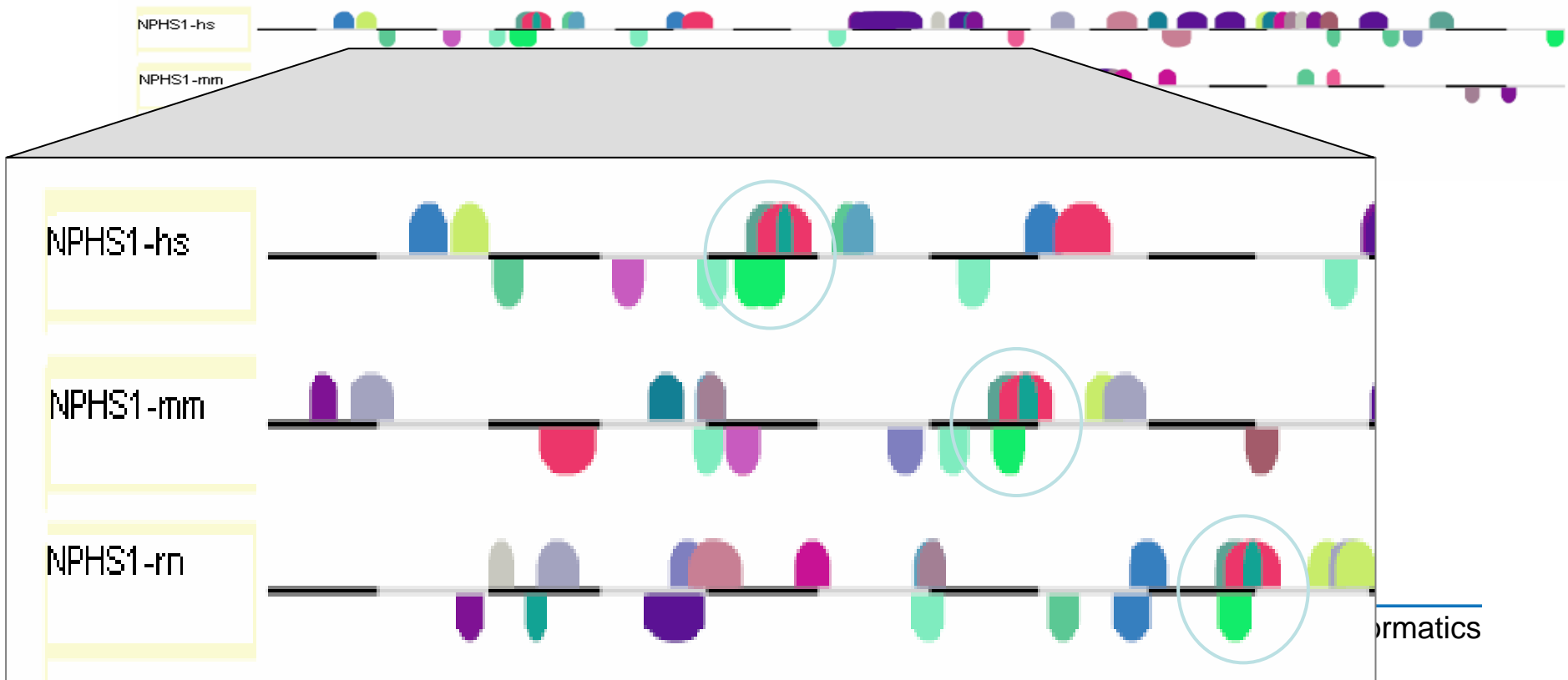
# Promoter modeling of glomerular filter proteins





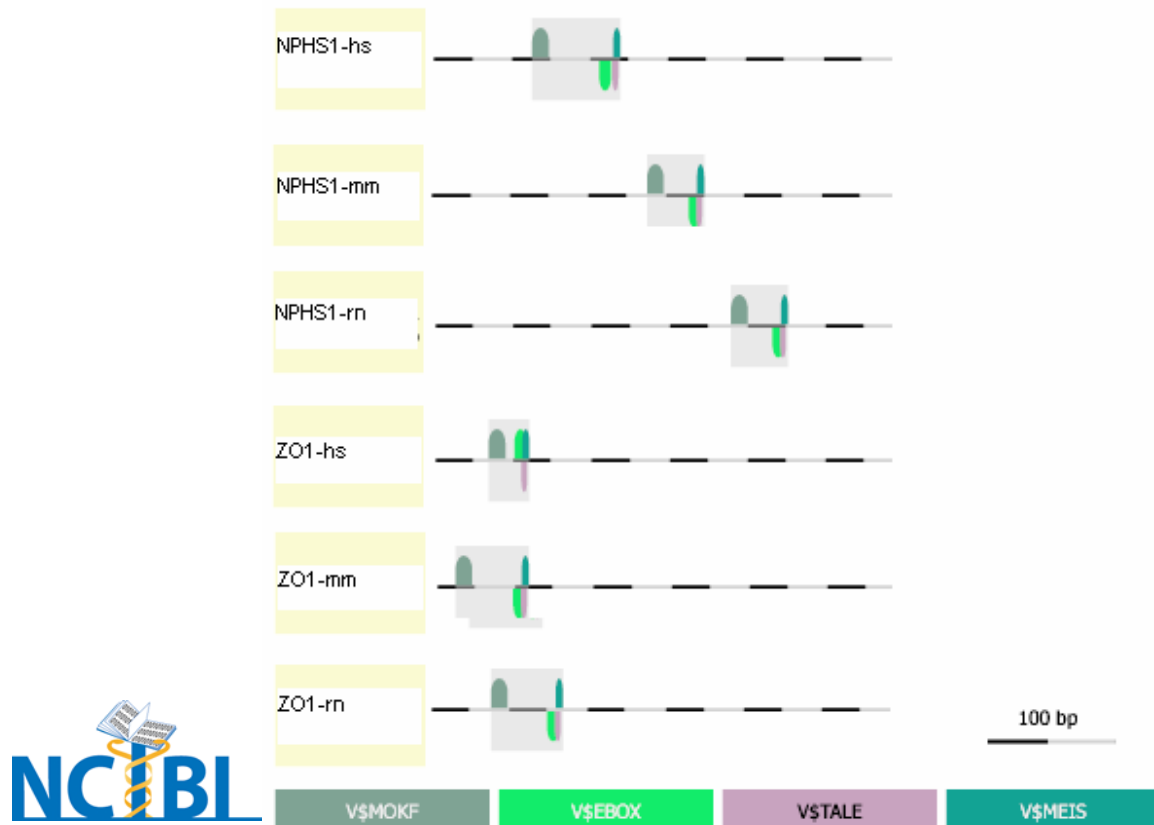
# Nephrin promoter modelling

- Phylogenetic footprinting:
  - Functional promoter elements should be evolutionary conserved
    - Identification of cross-species conserved domains in human, rat and mouse in the nephrin promoter



# Identification of conserved promoter modules

- Cross-species conserved nephrin module was searched in promoter database of slit diaphragm and podocyte associated molecules
- ZO-1 promoter showed conserved domain in all three species

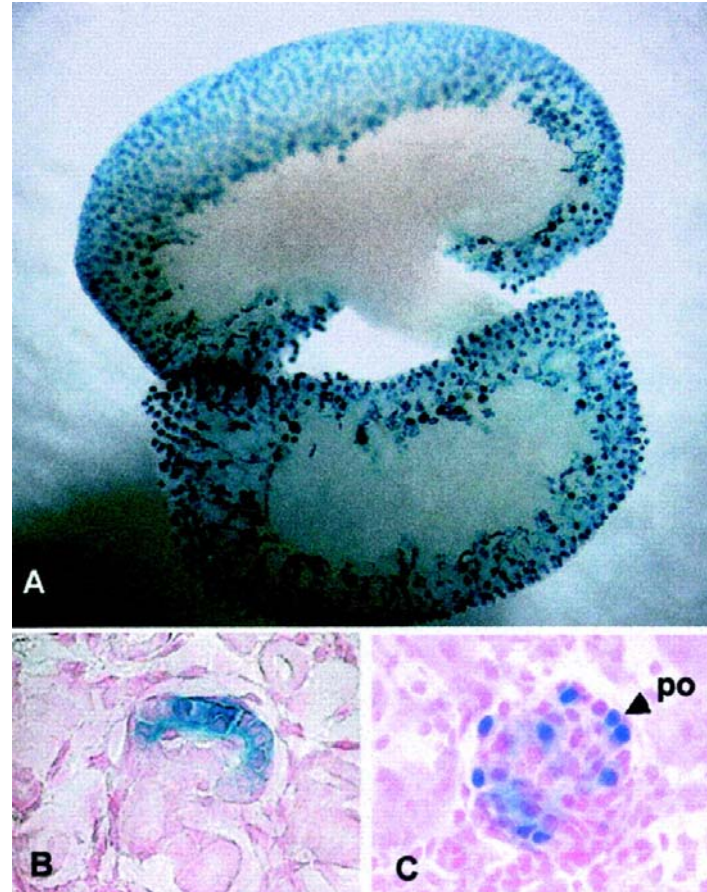


Cohen et al. Proc Natl Acad Sci USA, 2006  
Biomedical Informatics

# Conserved promoter module is a podocyte specific enhancer

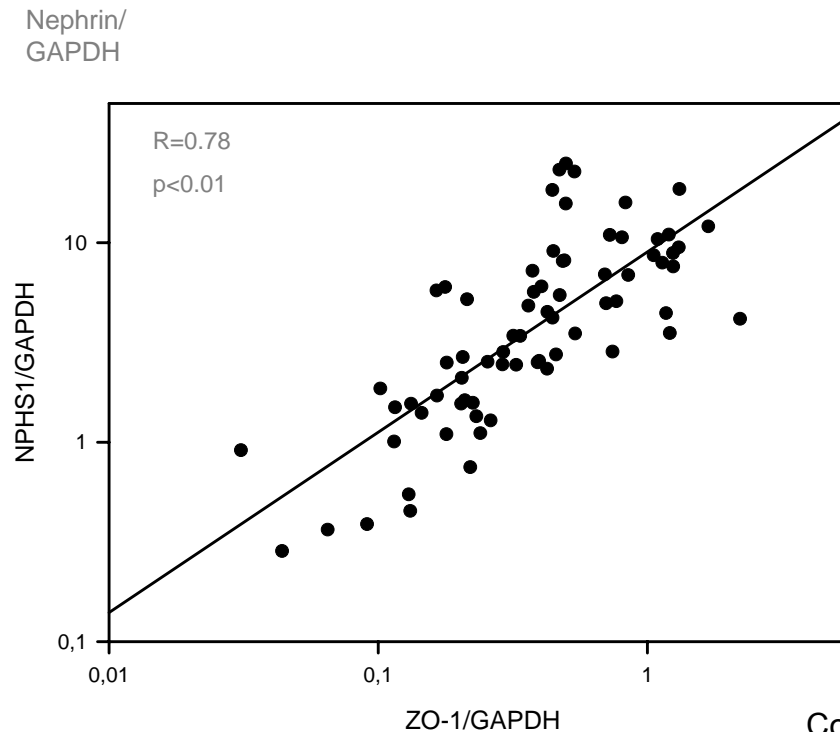
- WT-1 response element in Nephrin promoter
- Generation of transgenic mice with 186bp Nephrin-enhancer element targeting  $\beta$ -galactosidase expression to podocytes.

(Guo G et al. J Am Soc Nephrol 2004)



# Regulation of Nephrin and ZO-1 in podocytes in vivo

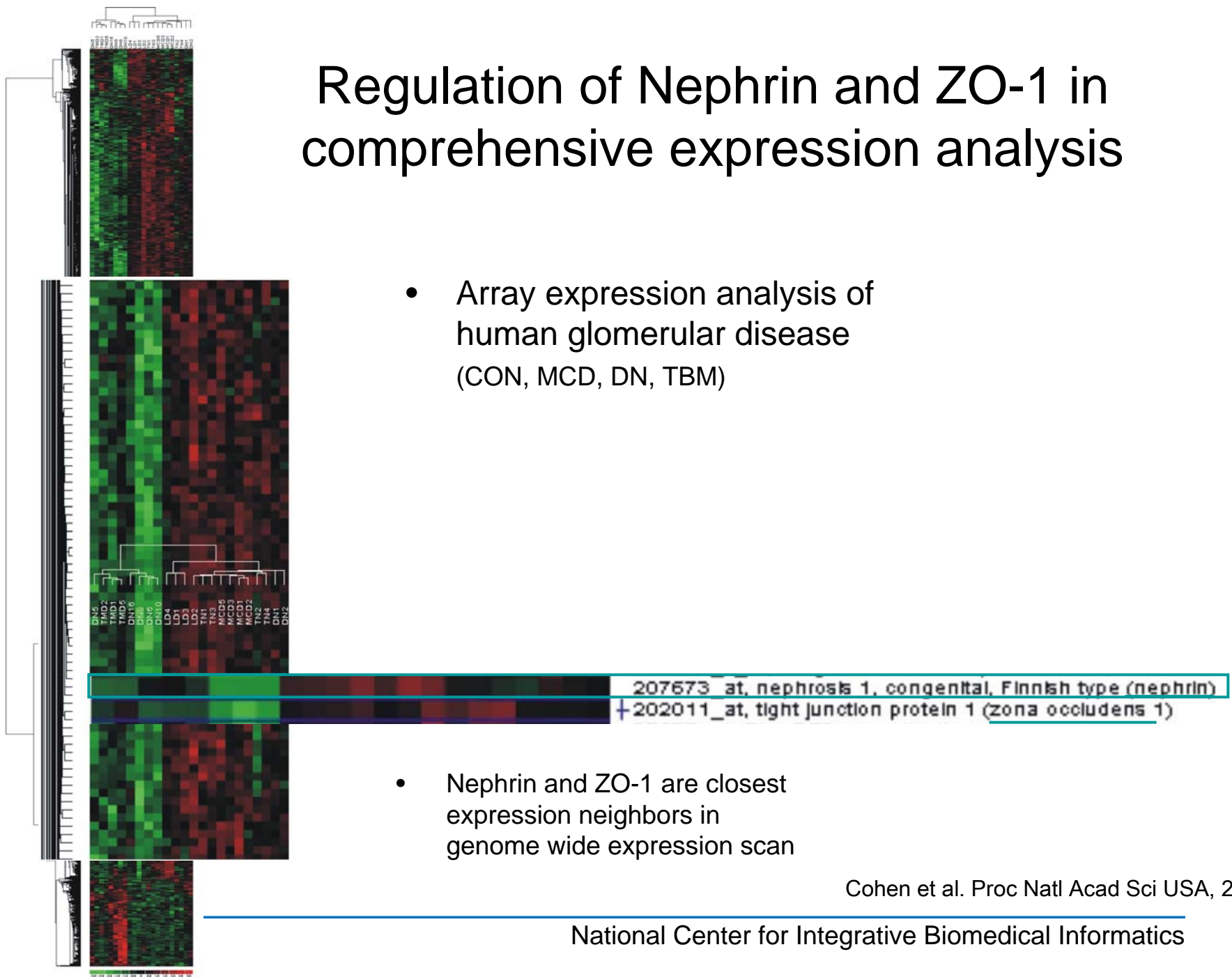
- Transcriptional co-regulation in human glomerular disease?
  - Microdissected human glomeruli obtained in the ERCB network
  - Proteinuric glomerular disease (n=76)
  - Nephrin and ZO-1 mRNA quantified using real-time RT-PCR



Cohen et al. Proc Natl Acad Sci USA, 2006

# Regulation of Nephrin and ZO-1 in comprehensive expression analysis

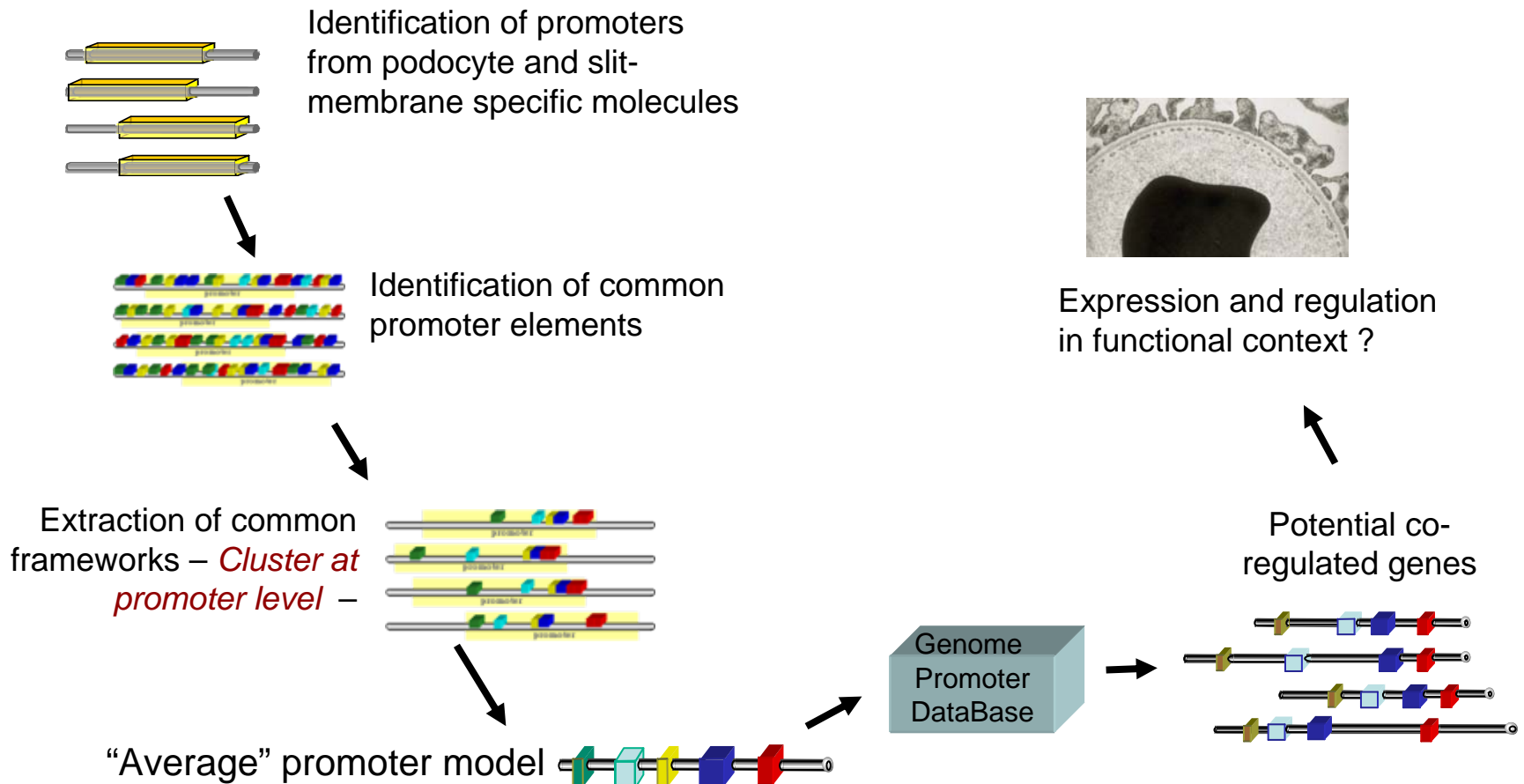
- Array expression analysis of human glomerular disease (CON, MCD, DN, TBM)



- Nephrin and ZO-1 are closest expression neighbors in genome wide expression scan

Cohen et al. Proc Natl Acad Sci USA, 2006

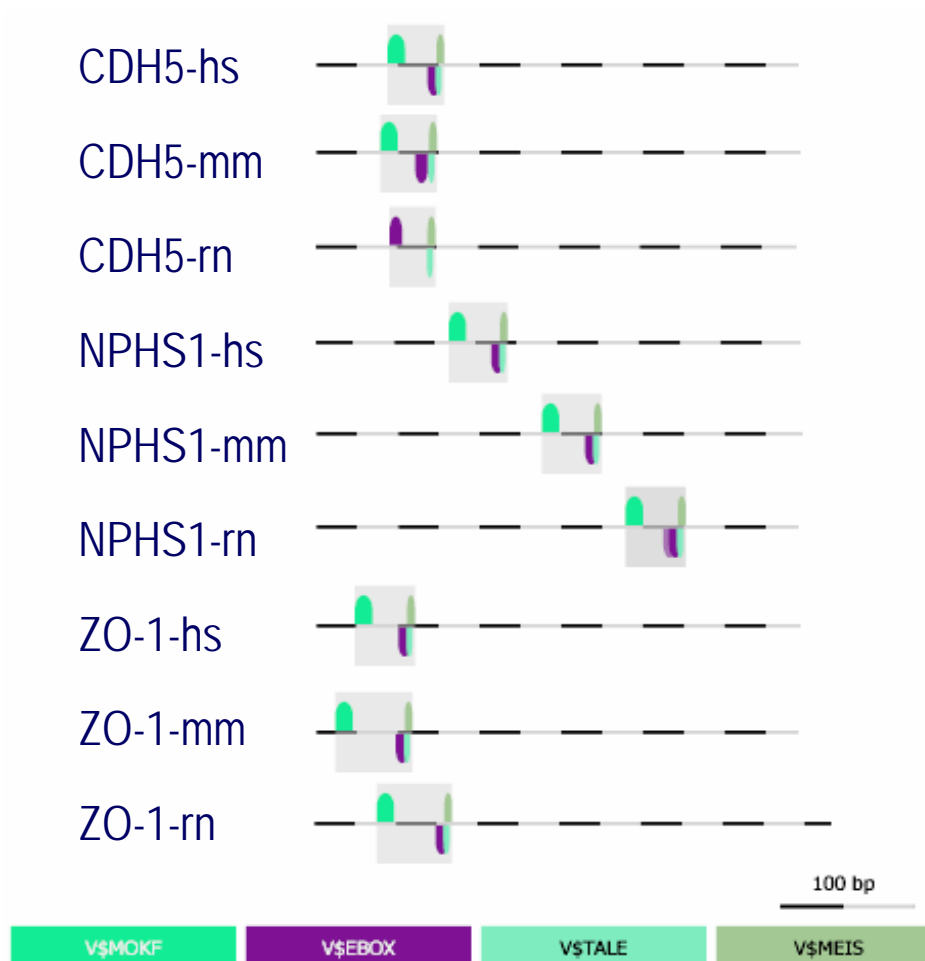
# Genome wide human promoter screen



# Genome wide human promoter screen

- Genomatix human promoter bank screened with the „NPHS1-ZO1-module“:
  - 79/50.145 of predicted promoters contained module (including ESTs and unknown genes)
  - 40/79 promoters of characterized genes
  - 6/40 modules showed cross species conservation
  - mRNA expression analysis:
    - 5/6 genes showed expression in
      - Glomeruli *ex vivo*
      - Podocytes *in vitro*

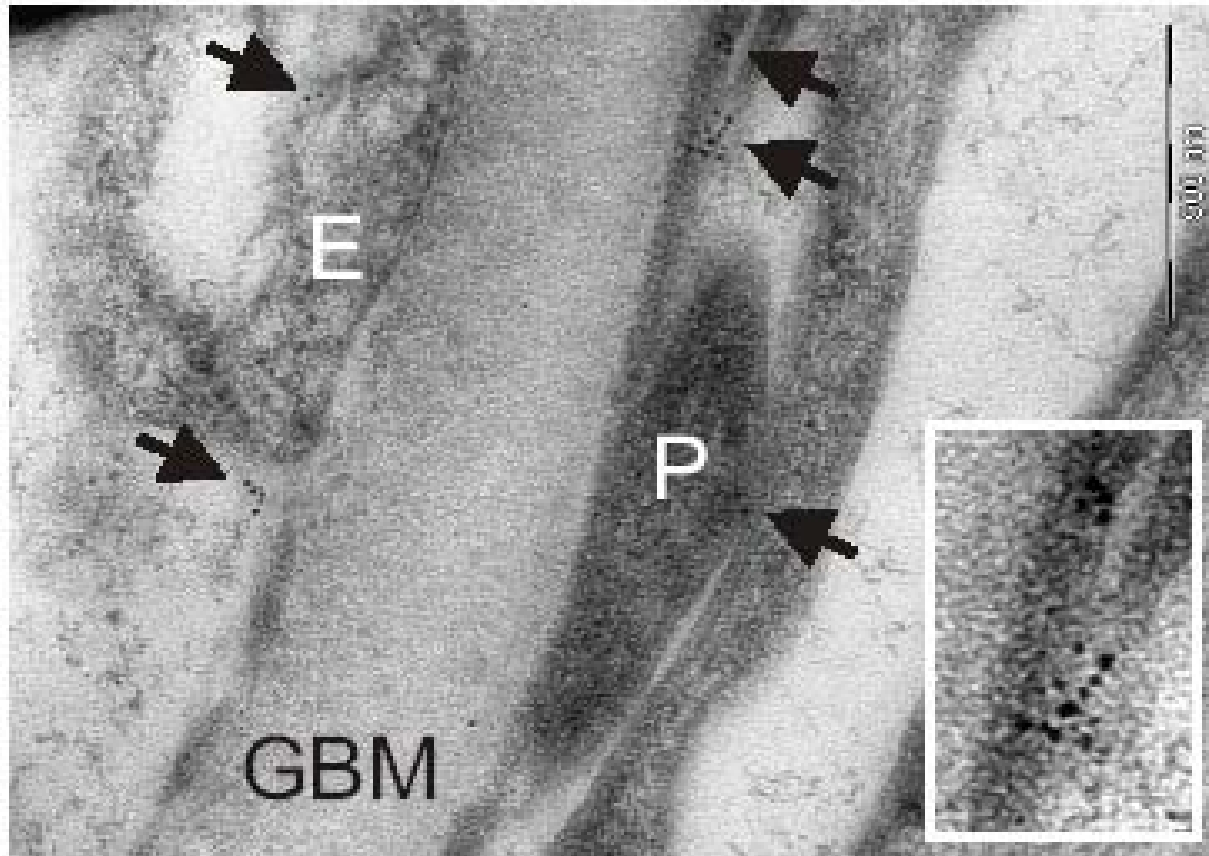
# Cadherin 5 contains ,nephrin-ZO-1‘promoter module





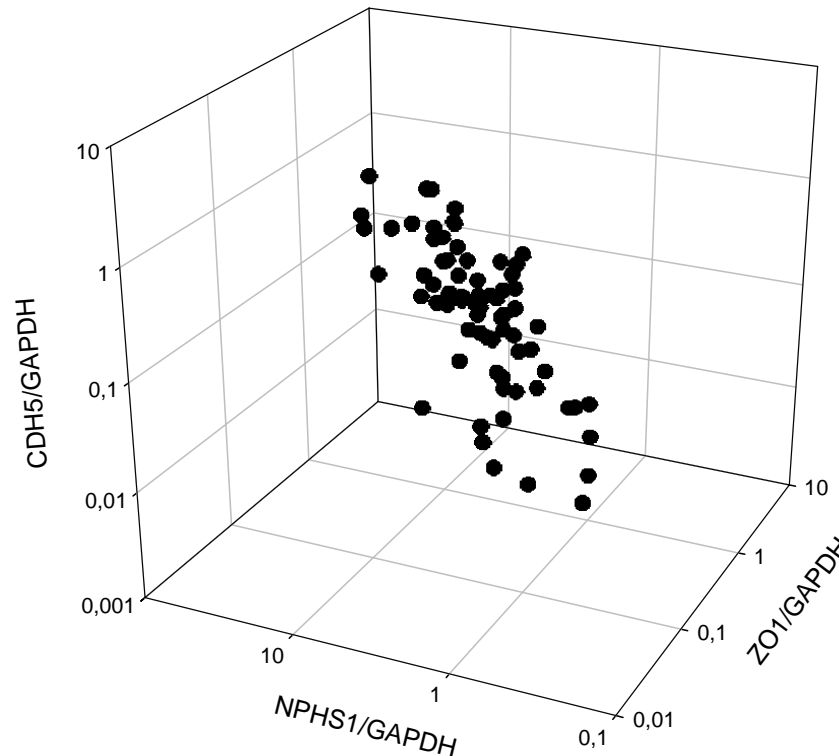
# Cadherin 5 expression in podocytes

Immunogold EM



# Co-Regulation of Cadherin 5, Nephrin and ZO-1 in human glomerular disease

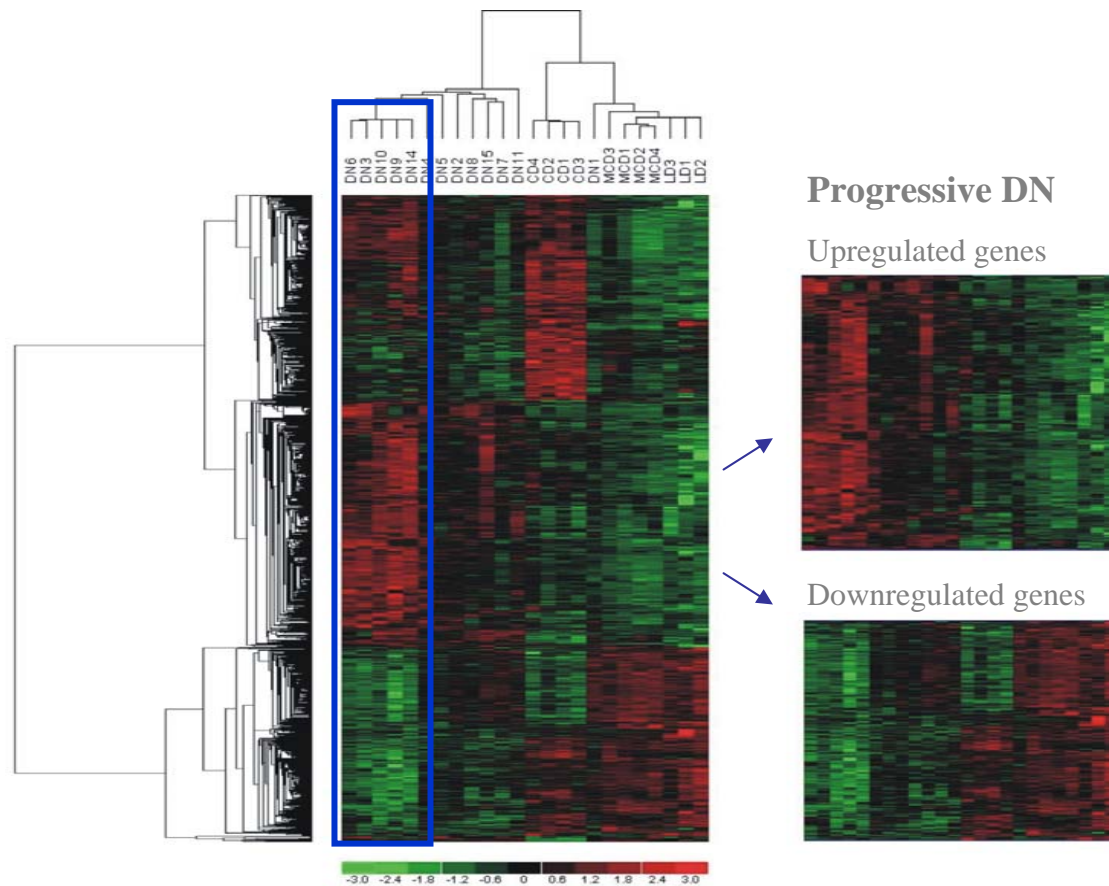
- Transcriptional co-regulation in human glomerular disease ?
  - 76 microdissected human glomeruli
  - Cadherin5, Nephrin and ZO-1 mRNA quantified using real-time RT-PCR



# 3. Extraction of diabetic disease markers and pathways

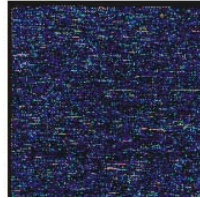
- Molecular diagnostic markers
- Transcriptional control of diabetic nephropathy
  - Transcriptional control mechanism of cell type specific gene expression
  - Identification of disease stage specific transcriptional control modules

# Integrative approach for transcriptional control in Diabetic Nephropathy



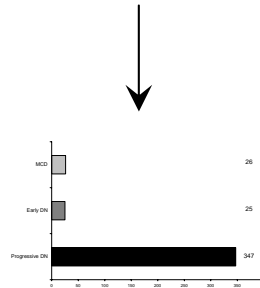
# Integrated analysis of prog. DN

Genome wide expression profile:  
Disease specific expression  
signature



*Differentially regulated mRNAs  
in DN vs control*

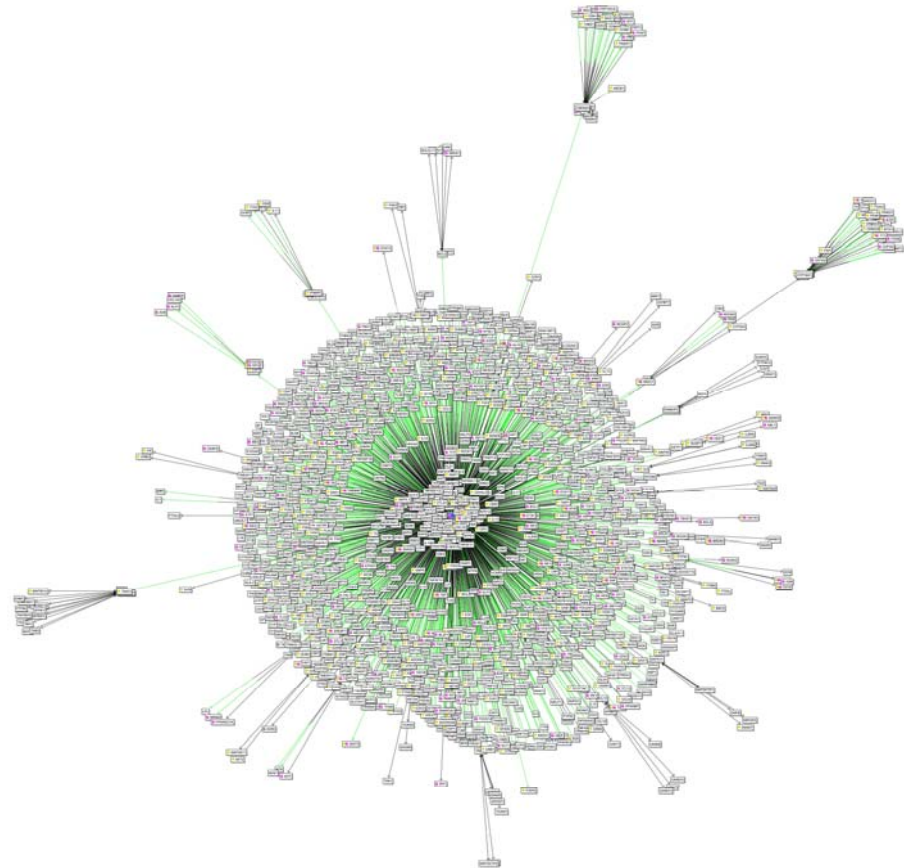
Defining functional categories  
of expression signature  
in Gene Ontology



*Ontology categories in prog DN:  
Inflammation-stress response*

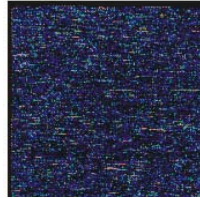
# Inflammation / stress response in Diabetic Nephropathy: Role of NF-kB?

- NLP: enrichment of regulated mRNAs co-cited with NF-kB
  - 1764 regulated mRNA:
    - co-citation on abstract level:  
NFkB - function word - gene:  
1208
    - NFkB promoter modules:  
977



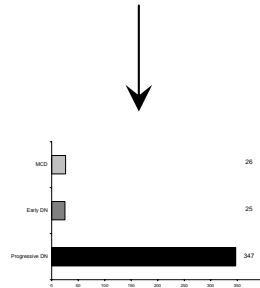
# Integrated analysis of prog. DN

Genome wide expression profile:  
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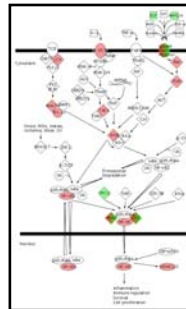
*Differentially regulated mRNAs  
in DN vs control*

Defining functional categories  
of expression signature  
in Gene Ontology



*Ontology categories in prog DN:  
Inflammation-stress response*

Selection of relevant pathway  
from GO category of interest:  
Pathway maps



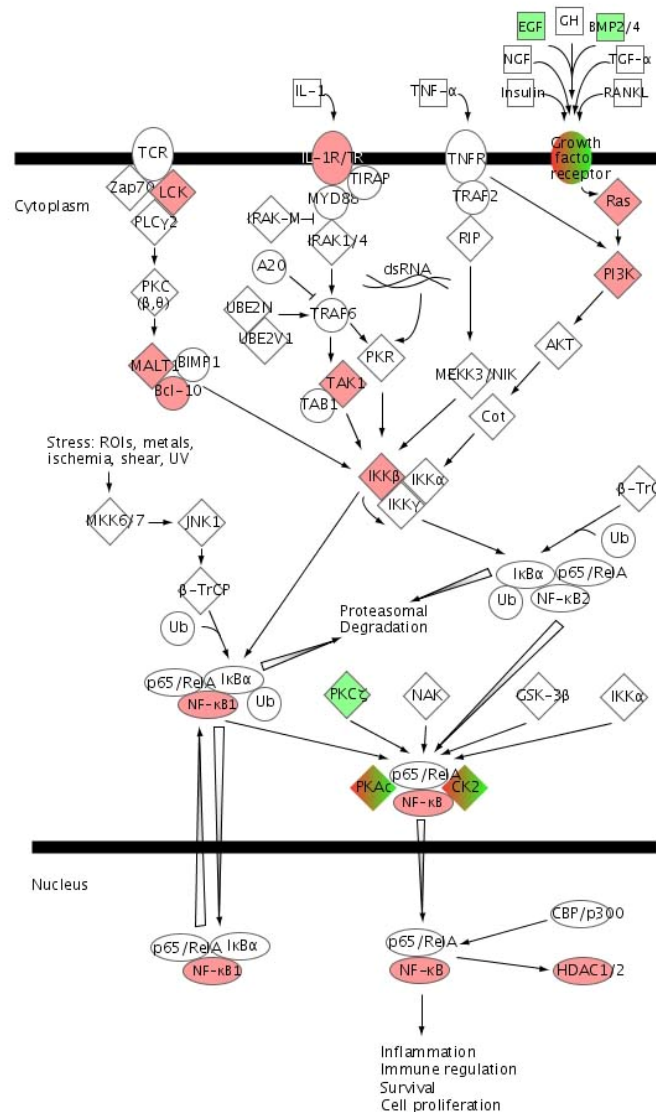
# Activation of NF-κB

Ligand

Receptor

Cytoplasmic activation cascade

NF-κB dependent transcriptional activation



27% of all elements regulated





Genome wide expression profile:  
Disease specific expression  
signature

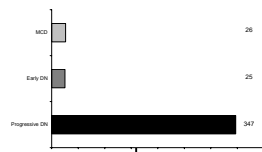
Defining functional categories  
of expression signature  
in Gene ontology

Selection of relevant pathway  
from GO category of interest:  
NPL / Pathway maps

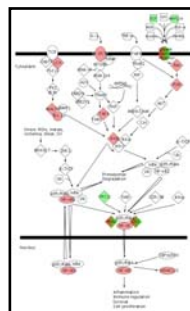
Prediction and evaluation of  
transcriptional consequences  
by activated pathway



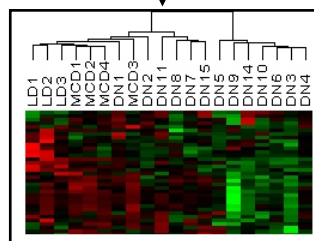
*Differentially regulated mRNAs  
in DN vs control*



*Ontology categories in prog DN:  
Inflammation-stress response*



*NF-κB pathway mapping in DN*

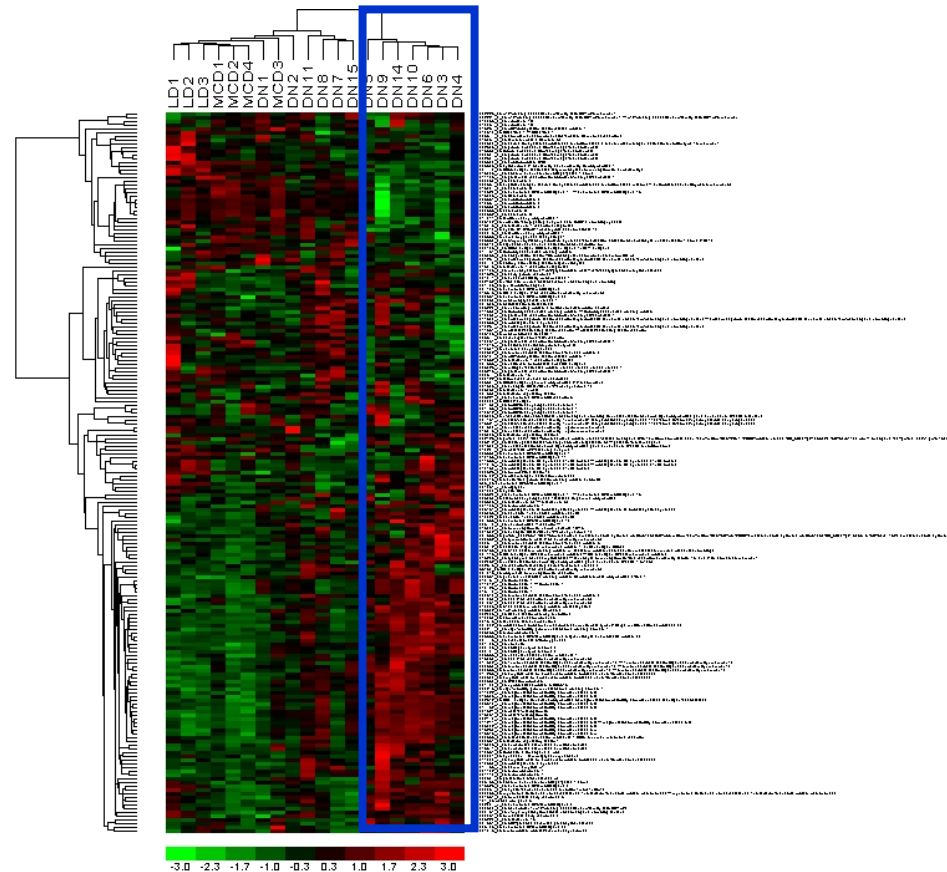


## NF-κB Pathway:

# Downstream Targets in progressive DN

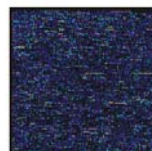
## Experimentally verified NF- $\kappa$ B dependent mRNAs:

- 232 NF-κB transcripts
  - 138 in renal tissue
    - mRNAs  $p < 0.05$ :  
54 (39%)

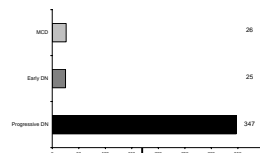


Genome wide expression profile:  
Disease specific expression  
signature

*Differentially regulated mRNAs in DN  
vs control*

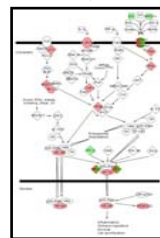


Defining functional categories of  
expression signature



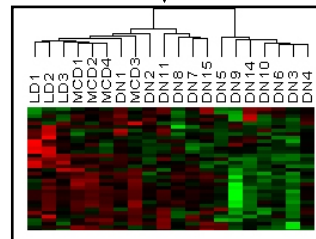
*Ontology categories in prog DN: Inflammation-  
stress response*

Selection of relevant pathway  
from GO category of interest:  
NLP / pathway maps



*NF- $\kappa$ B pathway mapping in DN*

Prediction and evaluation of  
transcriptional consequences  
by activated pathway



*Prog. DN shows sig. regulation of  
NF- $\kappa$ B dependent molecules,  
sufficient to define prog. DN*

Definition of shared  
promoter modules in pathway  
regulated mRNAs

Promoter module



# Established NF-kB modules in progressive DN

Experimentally verified modules	Up-regulated NF-kB target genes		Non-induced NF-kB target genes		All human promoter library	
	Number of matches	Probability (/10.000 bps)	Number of matches	Probability (/10.000 bps)	Number of matches	Probability (/10.000 bps)
AP1F_NFKB_01	0	0.000	1	0.111	53	0.015
AP1F_NFKB_02	1	0.164	2	0.222	41	0.012
AP1F_NFKB_03	4	0.657	1	0.111	145	0.041
<b>AP1F_NFKB_04</b>	<b>2</b>	<b>0.328</b>	<b>0</b>	<b>0.000</b>	<b>19</b>	<b>0.005</b>
AP1F_NFKB_EBOX_01	0	0.000	1	0.111	2	0.001
CEBP_NFKB_01	0	0.000	1	0.111	34	0.010
CEBP_NFKB_02	2	0.328	1	0.111	12	0.034
<b>CEBP_NFKB_04</b>	<b>2</b>	<b>0.328</b>	<b>0</b>	<b>0.000</b>	<b>111</b>	<b>0.032</b>
CEBP_NFKB_05	1	0.164	0	0.000	139	0.040
CEBP_NFKB_06	6	0.985	3	0.332	>1000	0.427
CEBP_NFKB_NFAT_02	0	0.000	1	0.111	204	0.058
CEBP_NFKB_STAT_01	1	0.164	0	0.000	1	0.000
CREB_NFKB_01	1	0.164	1	0.111	138	0.039
CREB_NFKB_03	1	0.164	0	0.000	127	0.036
GATA_GATA_NFKB_NFKB_01	1	0.164	0	0.000	1	0.000
IRFF_NFKB_01	1	0.164	0	0.000	106	0.030
IRFF_NFKB_03	0	0.000	2	0.222	70	0.020
NFKB_AP1F_01	4	0.657	3	0.332	>1000	0.582
NFKB_AP1F_SP1F_01	0	0.000	2	0.222	14	0.004
NFKB_CEBP_01	1	0.164	4	0.443	596	0.170
NFKB_CREB_01	7	1.149	4	0.443	>1000	0.584
NFKB_ETSF_01	0	0.000	1	0.111	54	0.015
<b>NFKB_IRFF_01</b>	<b>5</b>	<b>0.821</b>	<b>0</b>	<b>0.000</b>	<b>62</b>	<b>0.018</b>
NFKB_NFKB_01	1	0.164	0	0.000	16	0.005
NFKB_NFKB_02	0	0.000	1	0.111	10	0.003
NFKB_NFKB_03	0	0.000	1	0.111	48	0.014
NFKB_RBPF_01	3	0.493	6	0.665	344	0.098
NFKB_SORY_01	14	2.299	10	1.108	772	0.220
NFKB_SORY_02	10	1.642	4	0.443	350	0.100
NFKB_STAT_01	1	0.164	1	0.111	26	0.007



# NF- $\kappa$ B - IRF-1 promoter module

- Module can be tested for predictive power:
  - 9 genes reported to be regulated by NF $\kappa$ B-IRF-1 in vitro
- Test prediction for differential regulation in vivo:
  - Independent cohort of patients with progressive DN
  - qRT-PCR based assay:
    - 8/9 NF $\kappa$ B-IRF-1 dependent mRNAs sig. regulated

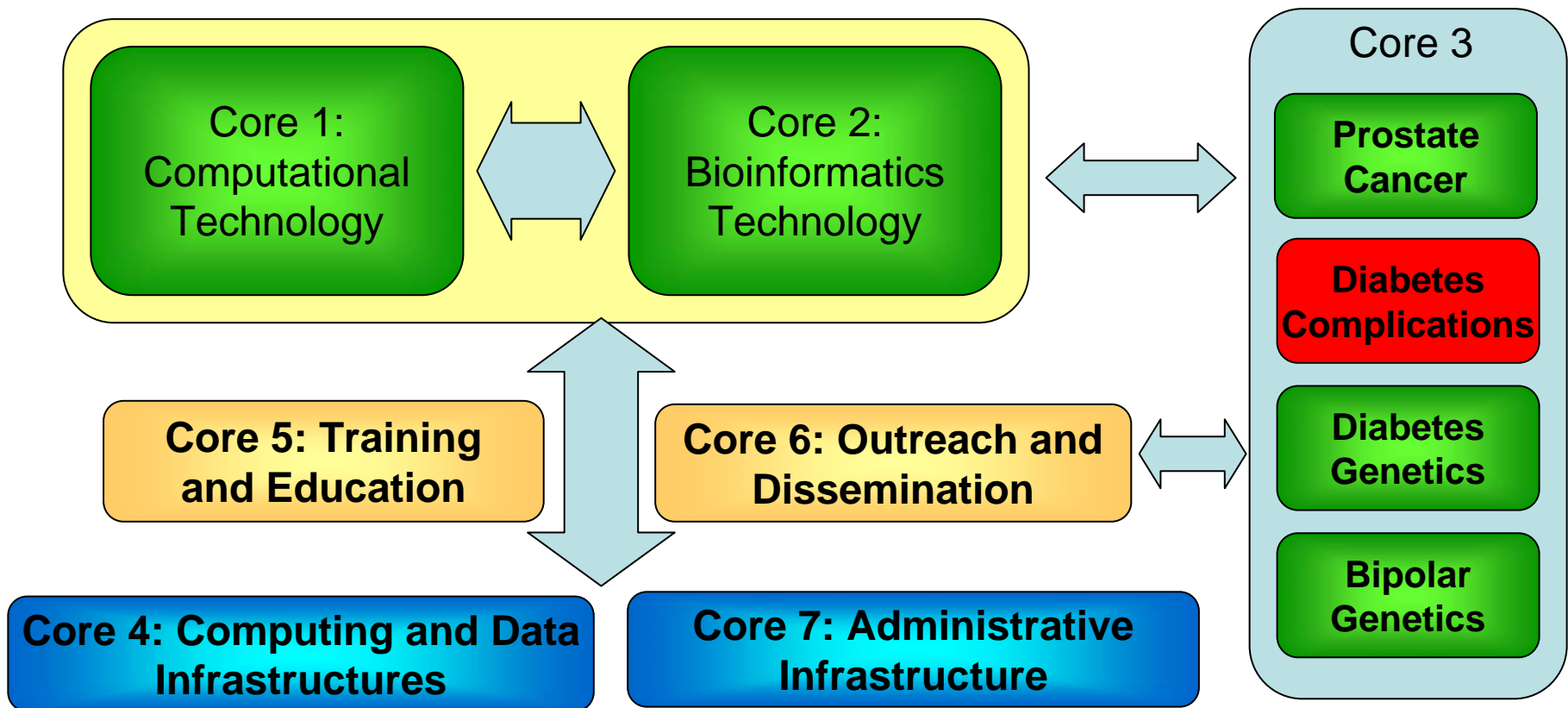
=> Specific inflammatory pathway in DN

# 4. Optimal utilization of expression data by research community

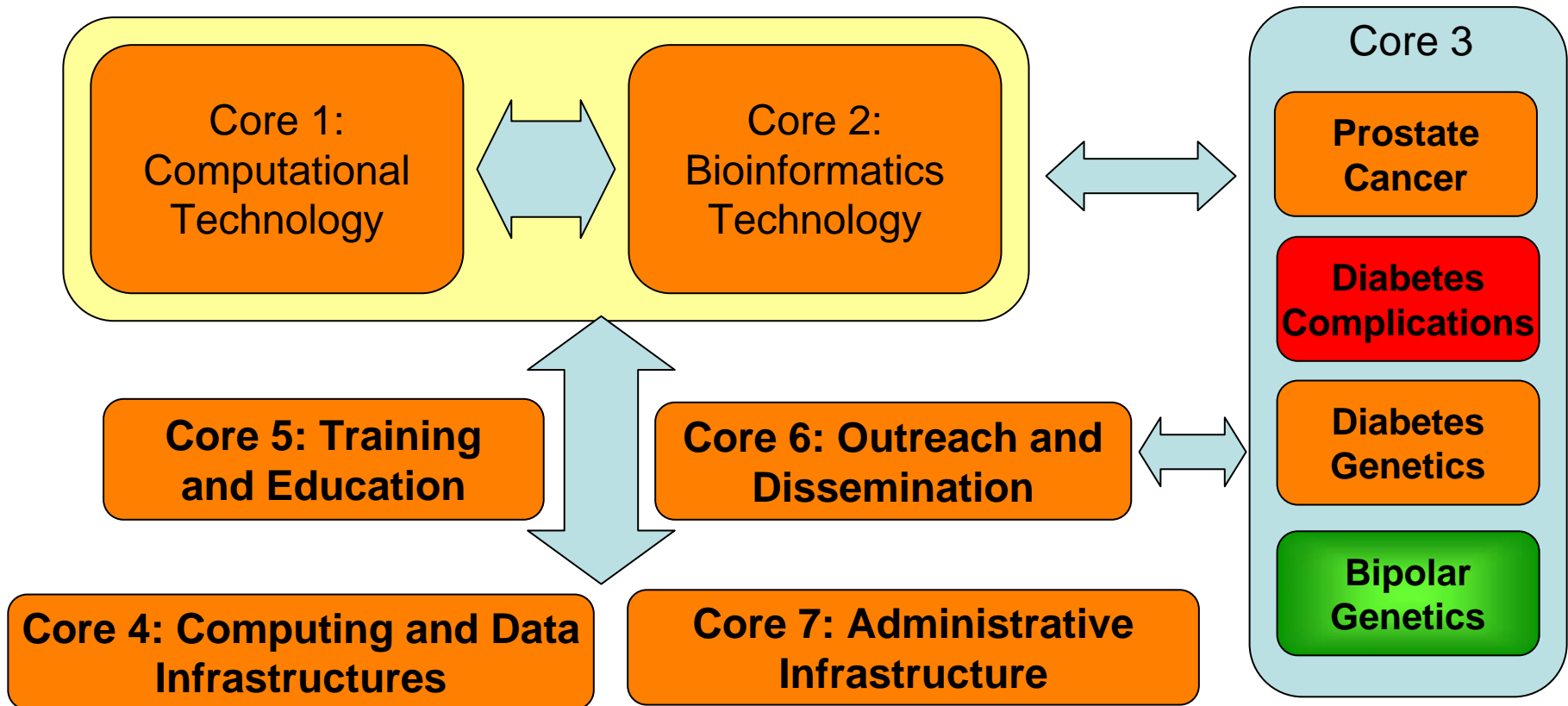
- Current strategy:
  - Direct supervised mining in specific research context:
    - Individual application of software tools
      - Labor and cost intense
  - Databases for integrative data mining
    - Tools in oncology (ONCOMINE, A.Chinnaiyan, D. Rhodes)
      - Data sets imported out of public domain or direct submissions
- Disease specific data mining tools:  
From Oncomine to Nephromine
  - 239 kidney arrays uploaded
    - Web-based interface with panel of tools and application for interactive search
  - Integration of published renal disease studies
    - 39 genome wide expression studies
  - System easy to navigate:
    - Non-expert users extract data on research focus in disease specific manner



# NCIBI Structure



# NCIBI Structure





# The network

## University of Michigan:

### *Internal Medicine*

Frank Brosius  
Sub Pennanthur  
Rodica Pop-Busui  
Anna Henger  
Simone Blattner  
Celine Berthier  
Pandu Rao  
Kathryn Lindblad

### *Neurology*

Eva Feldman  
Tim Wiggins

### *Rheumatology*

Joe McCune

### *Pathology*

Arul Chinnaiyan  
Paul Killen

Tom Giordano

### *Statistics*

Kerby Shedden

### *Chemical Engineering*

Peter Woolf

### *School of Public Health*

Laura Scott

Michael Boehnke

## University of Munich:

Clemens D. Cohen  
Peter J. Nelson  
Holger Schmid  
Dettef Schlöndorff

## Renal immunopathology, Milan:

Maria P. Rastaldi

## DKFZ, Heidelberg:

Hermann-Josef Gröne

## Genomatix GmbH, Munich:

Andreas Klingenhoff  
Thomas Werner

## Sanofi-Aventis Germany, Frankfurt:

Almut Nitsche  
Bodo Brunner  
Klaus-Peter Koller

## Renal biopsy bank:

P. Mertens, J. Floege, Aachen; A. J. Rees, Aberdeen; R. Nelson, Arizona; L. Gesualdo, F. P. Schena, Bari; H. Peters, H. H. Neumayer, Berlin; H.P. Marti, Bern; K Ivens, B. Grabensee, Düsseldorf; J. P. Doran, H. R. Brady, Dublin; F. Strutz, G. Müller, Göttingen; M. Zeier, Heidelberg; H. Holthoefer, Helsinki; J. Gerth, U. Ott, G. Wolf, Jena; K. Lemley, Los Angeles; F. Mampaso, A. Ortiz, Madrid; R.Langman, Melbourne; P. Rastaldi, G. D'Amico, Milano; C.Cohen, D.Schlöndorff, Munich; Y.Yasuda, Nagoya; B..Schroppel, New York; P. Rougier, P. Ronco, Paris; M. Merta, V. Tesar, Prague; B. Banas, B. Krämer, Regensburg; K. Blouch, B. Meyers, Stanford; H. Reich, Toronto; T. Risler, Tübingen; R. Oberbauer, D. Kerjaschki, Vienna; M. D. Mönks, C. Wanner, Würzburg; R. Wuetrich, Zurich,





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National Center for Integrative Biomedical Informatics

# Integrative analysis of diabetic end-organ damage

